DISCUSSION.

Assay results by the lethal frog method, which includes also the four-hour method, show that the International Standard digitalis leaves and the Canadian Standard leaves are very nearly equal to each other in activity, as they should be. An average of six tests shows the International Standard to be 107% of the Canadian Standard or that the latter is 93.5% of the former. This is a close enough agreement experimentally to consider them equal.

By the U. S. P. X one-hour method and against the official Ouabain standard the International Standard tincture averages 123% of the U. S. P. in five tests and the Canadian Standard tincture averages 128% of the U. S. P. or nearly the same as the B. P. Since each of these averages contains one or two high results obtained on winter frogs (Jan. 1933) it would seem that the B. P. and Canadian Standard tinctures of digitalis are from 20% to 25% more active than the U. S. P. Standard tincture and that conservatively the difference might be considered as being fully 20%.

By the cat method (two tests) the International Standard seems to be fully 90% of the standard proposed by Hatcher for Tincture of Digitalis but this series of tests is not large enough to prove much except that the B. P. 1932 Standard Tincture of Digitalis is about equal to the Hatcher Standard.

CONCLUSIONS.

1. The standard tincture of digitalis of the British Pharmacopœia, 1932 revision, is fully 20% more active than the U. S. P. X Standard. This difference is significant and is difficult to determine because of the recommended use of different standards and methods of bioassay.

2. The B. P. 1932 Standard tincture and the Canadian Standard, 1928 regulations, Food and Drugs Act are equal, within the limits of experimental error of the methods of assay, as they should be.

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PHYTOCHEMICAL NOTES.*

NO. 110. THE STEROLS FROM STRAMONIUM SEED.[1]

BY OLE GISVOLD.

The unsaponifiable material obtained by Ralph Clark in his study of the fatty acids of stramonium seed (1) was heated under a vacuum on a water-bath for a half day. It was then allowed to stand for several days in a vacuum desiccator in order to remove any traces of moisture present. Ten grams of this material (2) were dissolved in sufficient alcohol to make 150 cc. of solution. Two and one-half grams of digitonin, Merck, were dissolved in 250 cc. 90 per cent alcohol. Both

* From the laboratory of Edward Kremers.

[1] Scientific Section, A. PH. A., Toronto meeting, 1932.

solutions were heated to 65° and the digitonin solution was added to the solution of the unsaponifiable material. An immediate separation of the digitonide took place. The mixture was allowed to stand over night and filtered by means of suction. The precipitate was suspended in alcohol, filtered off again and the precipitate washed several times with alcohol and ether.

A test made with the first mother liquor gave a precipitate with digitonin. Therefore, 200 cc. of 1 per cent digitonin solution were added to the mother liquor in the manner previously described. There was no immediate precipitate, but upon cooling the digitonide crystallized out. After standing over night the precipitate was treated as previously described. It is a distinct advantage to thoroughly wash the digitonide as previously described, otherwise oil will be encountered in the crystallization of the sterol. As the solubility of the digitonide is only 0.014 (3) Gm. in 100 cc. alcohol at 18°, the loss of sterol cannot be appreciable. The second mother liquor was concentrated to half its volume, when upon cooling, a third though small quantity of digitonide settled out.

Dried in a desiccator the following weights of the precipitates were determined:

I	2.9809 Gm.
II	1.9030 Gm.
III	0.4014 Gm.
Total	5.2853 Gm. corresponding to 1.321 Gm. of sterol or 13.21 per cent of the unsaponifiable material.

Precipitate No. I, placed in a thimble, was extracted for ten hours with xylene by continuous percolation. The xylene of the resulting solution was evaporated on a water-bath under reduced pressure. The residue left in the flask was then dissolved in ether, alcohol added to the solution and the ether boiled off. A small quantity of charcoal was added to the alcoholic solution and the mixture boiled gently for a few moments with continuous agitation, and then filtered. The alcoholic filtrate of the sterol was reheated and just enough water added until the temporary turbidity thus produced threatened to become permanent. Upon cooling beautiful opaque leaflets were obtained which upon recrystallization, melted at 134° to 135°. The mother liquor yielded crystals m. p. at 127° to 128°. The first crop of crystals was acetylated by boiling gently for one hour with an excess of acetic acid anhydride. Upon recrystallization the acetate melted at 131° to 132°. The acetate was saponified (4) and upon recrystallization the regenerated sterol melted at 137° to 138°.

When treated according to Windaus u. Hauth, the acetate gave no precipitate, indicating the absence of stigmasterol (7).

Burian's sitosterol (5) melts at 137° to 138°, its acetate at 127° to 128°.

Anderson and Shriner (6) have shown that the sterol obtained from corn oil m. p. 137° to 138° can be resolved into γ, β and α sitosterol.

Anderson and Nabenhauer (8), report a highly purified sitosterol m. p. 138° to 139°, the acetate melting at 130° to 131°.

Digitonide precipitates II and III were combined and were subjected to the same treatment as No. I. The recovered sterol crystals were combined with those that had previously been obtained as a second drop of the first batch with a m. p.

127° to 128° because of similarity of m. p. The acetate obtained upon recrystallization melted at 120° to 121°.

The material marked "III A" by Clark was recrystallized. Beautiful almost transparent leaflets were obtained which melted at 135°. Even after drying at 100° over P_2O_5 in a vacuum the melting point was not changed.

The remainder of the unsaponifiable material which amounted to 53 Gm. was treated with digitonin in the manner previously described. The sterol thus obtained exhibited the same physical properties as that obtained before, *i. e.*, the free sterol melted at 138°, and the acetate melted at 131° to 132°.

A mixed melting point was made with the sterol obtained from digitalis seed and no change in the melting point could be noticed. Evidently the two sterols are identical.

The sterol acetate was saponified with alcoholic potassium hydroxide (half normal) by refluxing for one and one-half hours. The excess alkali was back titrated with half normal hydrochloric acid. The values thus obtained indicate that the sterol has the composition $C_{26}H_{43}OH$.

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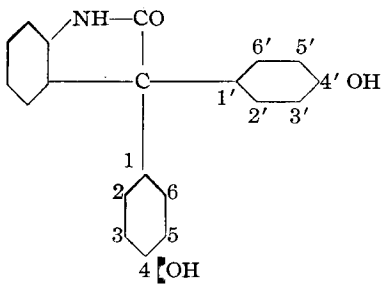
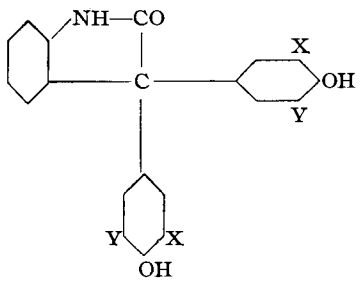
MERCURATED SUBSTITUTION PRODUCTS OF DIPHENOL ISATIN.*

BY S. E. HARRIS AND W. G. CHRISTIANSEN.

The results obtained during our study of mercury derivatives of phthaleins derived from *o*-hydroxy diphenyl (1) suggested that therapeutically valuable mercury compounds should be found among compounds of similar general structure. Diphenol isatin (I) was selected as the parent substance for the investigation and a number of mercury derivatives of the substituted products (II) (Y = NO_2 or Br; X = H, C_6H_5, CH_3, NO_2 or Br) were prepared.



(I)



(II)

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* Scientific Section, A. PH. A., Toronto meeting, 1932.